ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
Η ΔΙΑΤΡΟΦΗ ΣΤΗΝ ΥΓΕΙΑ ΚΑΙ ΣΤΗ ΝΟΣΟ

ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

Βιταμίνη Β12 και σακχαρώδης διαβήτης κύησης

Κουρόγλου Ελένη
Ιατρός

ΤΡΙΜΕΛΗΣ ΣΥΜΒΟΥΛΕΥΤΙΚΗ ΕΠΙΤΡΟΠΗ

Μπαργιώτα Αλεξάνδρα, Επίκουρος Καθηγήτρια Παθολογίας-Ενδοκρινολογίας (Επιβλέπουσα Καθηγήτρια)

Δαπόντε Αλέξανδρος, Καθηγητής Μαιευτικής-Γυναικολογίας (Μέλος Τριμελούς Επιτροπής)

Αναγνωστής Παναγιώτης, Ενδοκρινολόγος-Διαβητολόγος, Διδάκτωρ Ιατρικής Σχολής ΑΠΘ (Μέλος Τριμελούς Επιτροπής)

Λάρισα, 2018

ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΣΧΟΛΗ ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑ ΙΑΤΡΙΚΗΣ
ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ
Η ΔΙΑΤΡΟΦΗ ΣΤΗΝ ΥΓΕΙΑ ΚΑΙ ΣΤΗ ΝΟΣΟ

Vitamin B12 and gestational diabetes mellitus

Table of Contents

1. Abstracts in English and Greek

1.1 Abstract in English

Objective/Design: Gestational diabetes mellitus (GDM) has been associated with serious complications for both the pregnant woman and the newborn. Vitamin B12 is implicated in some important metabolic procedures such as methylation and one carbon cycle and its deficiency can cause serious health problems, such as hyperhomocysteinemia, defective synthesis of neurotransmitters and fatty acids and more. However, it is not known if there is a link between vitamin B12 deficiency and the risk of GDM. The purpose of this study is to systematically investigate and meta-analyze the evidence that exists up to date for this association.

Methods: A comprehensive research was conducted in PubMed, Scopus and Cochrane Central Register of Controlled Trials up to November 30th, 2018. Data are expressed as odds ratio (OR) with 95% confidence intervals (CI). The I^2 index was employed for heterogeneity.

Results: Six studies (n=1,810 pregnant women, 309 GDM cases) fulfilled eligibility criteria for qualitative and two studies for quantitative analysis. Women with vitamin B12 deficiency were at higher risk for developing GDM when compared with those who were vitamin B12 sufficient: OR 1.81 (95% CI, 1.25-2.63, I^2: 0%). Due to the small number of studies, the role of potential confounders could not be clearly estimated.

Conclusions: Vitamin B12 deficiency seems to be associated with increased risk of GDM. The pathogenetic mechanisms for this association need to be clarified in future studies.

Keywords: vitamin B12, gestational diabetes mellitus, pregnancy, diabetes, vitamin B12 deficiency

1.2 Abstract in Greek

Αντικείμενο/Σχεδιασμός έρευνας: Ο σακχαρώδης διαβήτης κύησης (GDM), έχει συνδεθεί με σοβαρές επιπλοκές τόσο για τη γυναίκα που κυοφορεί, όσο και για το νεογνό. Η βιταμίνη B12 εμπλέκεται σε σημαντικές μεταβολικές διεργασίες όπως η μεθυλίωση και ο κύκλος του φυλλικού οξέως και η έλλειψή της μπορεί να προκαλέσει σοβαρά προβλήματα υγείας, όπως υπερομοκυστεϊναιμία., ελαττωματική σύνθεση νευροδιαβιβαστών και λιπαρών οξέων και άλλα. Ωστόσο, δεν είναι γνωστό αν υπάρχει κάποια σύνδεση μεταξύ της έλλειψης βιταμίνης B12 και του σακχαρώδη διαβήτη κύησης. Ο σκοπός αυτής της μελέτης είναι να γίνει συστηματική ανασκόπηση και μετα-ανάλυση των δεδομένων που υπάρχουν μέχρι σήμερα για τη πιθανή συσχέτιση των δύο παραγόντων.

Μέθοδος: Η έρευνα πραγματοποιήθηκε μέσα από τις βάσεις δεδομένων PubMed, Scopus, Cochrane Central Register Of Controlled Trials μέχρι και τις 30 Νοεμβρίου 2018. Τα δεδομένα παρουσιάστηκαν ως αναλογία πιθανοτήτων (OR) και 95% διάστημα εμπιστοσύνης (95% CI). Ο δείκτης I^2 χρησιμοποιήθηκε για την ετερογένεια των αποτελεσμάτων.

Αποτελέσματα: Έξι μελέτες συμπεριλήφθηκαν στη συστηματική ανασκόπηση και δύο μελέτες από αυτές παρείχαν τα απαραίτητα δεδομένα ώστε να μετά-αναλυθούν. Συνολικά, 1810 έγκυες γυναίκες μελετήθηκαν, με 309 περιπτώσεις διαβήτη κύησης. Οι γυναίκες με ανεπάρκεια βιταμίνης B12 είχαν μεγαλύτερο ρίσκο εμφάνισης διαβήτη κύησης: OR 1,81 (95% CI 1.25-2.63), Heterogeneity (I2) 0%. Εξαιτίας του μικρού αριθμού των μελετών που υπάρχουν μέχρι σήμερα, ο ρόλος των παραγόντων που θα μπορούσαν να επηρεάσουν τη συσχέτιση δεν μπόρεσε να εκτιμηθεί επακριβώς.

Συμπέρασμα: Η ανεπάρκεια της βιταμίνης B12 φαίνεται να σχετίζεται με αυξημένο κίνδυνο σακχαρώδη διαβήτη κύησης. Οι παθογενετικοί μηχανισμοί για αυτή τη συσχέτιση θα πρέπει να διευκρινιστούν σε μελλοντικές μελέτες.

Λέξεις κλειδιά: βιταμίνη B12, διαβήτης κύησης, εγκυμοσύνη, διαβήτης, ανεπάρκεια βιταμίνης B12

2. Introduction

The term ¨diabetes mellitus¨ (DM), describes a multifactorial disorder of carbohydrate, fat and protein metabolism, resulting from a defect in insulin secretion, action or both, with subsequent long-term health consequences, due to multi-organ failure (1). The term ¨gestational diabetes mellitus¨ (GDM), describes a state of carbohydrate intolerance of variable severity with onset of recognition during pregnancy. The definition is applied whether insulin or diet modification is used for treatment and whether this condition persists after pregnancy (2).

Therefore, women who become pregnant and who are known to have DM which antedates pregnancy do not have GDM, but 'DM and pregnancy' and should be treated accordingly before, during and after pregnancy (1).

GDM is a topic of major interest for many reasons. First it is a very common condition affecting up to 16% of women in high-risk populations (3). Second, it can lead to adverse pregnancy outcomes, as it affects both mother and fetus. Third, appropriate treatment may improve maternal health and quality of life and can also diminish the perinatal morbidity/mortality (4).

Diabetogenic effect of pregnancy is thought to play an important role in the development of GDM, where human placental lactogenic hormone (HPL) secreted from placenta during pregnancy results in insulin desensitization, leading to physiological increases in blood glucose levels, particularly during the last two trimesters of pregnancy (5).

The increase in growth hormone, cortisone, estrogen and progesterone concentrations during pregnancy also contribute in insulin resistance (6, 7). Furthermore, maternal age, ethnicity, genetics, polycystic ovary syndrome, hypertension and obesity are associated with higher risk of GDM (8, 9).

Over the last 20 years, the rates of obesity have doubled and this can be measured by the rise in the body mass index (BMI) in the general population which is doubling (7.6% to 15.6%) and the proportion of obese women at the time of conception which is also increasing (10). These rising rates of adiposity have led researchers to investigate if the paradox of nutritional deficiencies in those who are overweight or obese, may have implications in maternal health and the development of the embryo as a potential consequence of this phenomenon (11).

The micronutrients vitamin B12 and folate are necessary in order to support the increased demands of the fetus during pregnancy. Both vitamins are involved in metabolic processes such as the methylation process, the one carbon metabolism as well as epigenetic processes and DNA repair. Deficiency of these essential vitamins lead to megaloblastic anemia, growth retardation in utero, birth defects and neurocognitive disorders in the off-spring (12).

The primary aim of this systematic review was to investigate the relation between B12 deficiency during pregnancy and the risk of GDM. Secondary aim was the effect of BMI and other factors on this association.

3. General part

3.1 Gestational diabetes mellitus (GDM)

3.1.1 Classification

According to the World Health Organization (WHO), DM is classified into four categories: type 1 DM (T1DM), type 2 DM (T2DM), other rare types of DM and GDM. T1DM is characterized by autoimmune destruction of β-cells of the pancreas, which has been linked to genetic susceptibility, a diabetogenic trigger and/or exposure to a driving antigen (13). T2DM is characterized by insulin resistance and insulin deficiency. As far as the rare types of DM are concerned, they include the genetic defects of β-cell function or insulin action, diseases of the exocrine pancreas and drug-induced DM. GDM is considered as a fourth type, characterized by glucose intolerance that appears or is recognized for the first time during pregnancy (14, 15).

3.1.2 Pathophysiology

Adaptation of the maternal metabolism during pregnancy involves a number of physiological changes such as a greater fall in plasma glucose and amino acids and a greater rise in free fatty acids after an overnight fasting compared with the non-pregnant state (‘accelerated starvation’), which are associated with insulin resistance. In later pregnancy, a progressive rise in postprandial glucose and its associated insulin response, associated with decreased insulin sensitivity, parallels the growth of the fetal placental unit and rapidly reverses after delivery (16). GDM shares many pathophysiological characteristics with T2DM, most importantly insulin resistance (16).

The following figure shows the association between GDM and T2DM. The horizontal axis represents time and the vertical axis represents insulin production and/or action. The green line represents a woman who will never develop either GDM or T2DM (“control”). Although the production and action of insulin declines with age, she always remains above the threshold of developing clinical DM. During pregnancy, this woman develops insulin resistance, mainly due to the placental hormone production which antagonizes insulin. Nevertheless, she will not develop GDM, as the pancreas has functional reserves and is capable of producing larger amounts of insulin. Normally, after delivery, insulin production and action return to the pre-pregnancy levels. On the other hand, the red line represents a woman who will develop GDM, and possibly T2DM (“patient”). She is probably genetically predisposed to T2DM and/or has adopted a suboptimal lifestyle (e.g. obesity, lack of exercise). Although her insulin action is already at the lower level

compared with the control, she has not yet developed T2DM, being above the relevant threshold. During pregnancy, a third factor, insulin resistance, is added to genetic predisposition and suboptimal lifestyle. As a consequence, she develops GDM, since the pancreas, although producing larger amounts of insulin, does not have adequate functional reserves to completely overcome insulin resistance. Immediately after delivery, the placenta is removed and insulin production and action return to the pre-pregnancy level (17).

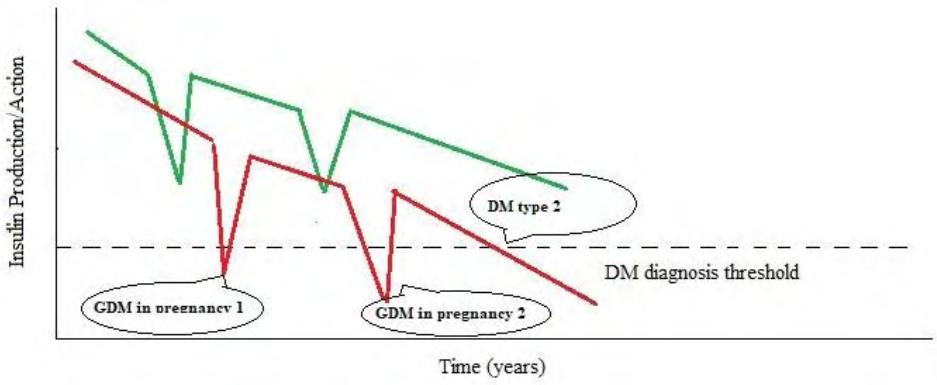

Figure 1: The relation of GDM and DM, adapted by Poulakos et al., 2015 (17)

3.1.3 History

Gestational diabetes mellitus has been an interesting topic since as early as 1882, that J. Matthews Duncan has observed that diabetes may appear during pregnancy, but will eventually disappear after delivery (18). In the 1950s, W.P.U Jackson noticed a high likelihood of previous stillbirth and fetal macrosomia in women with diabetes and in 1957 Elsie Reed Carrington et al. used the term ‘gestational diabetes’ (18).

3.1.4 Risk factors

GDM carries significant risks for both mother and fetus, being a serious public health issue, so it is important to understand the risk factors paying a special attention to the modifiable ones, that contribute to its development. By modifying these risk factors the intrauterine environment will be improved, which in turn may lower the risk of GDM- related adverse health outcomes for both women and off spring. Well - documented risk factors include advanced maternal age, family history of DM, previous pregnancy complicated with GDM, non-Caucasian race, increased body weight and cigarette smoking (19, 20).

In addition, lifestyle intervention before and during pregnancy seem to affect the risk of GDM. Conventionally, promoting physical activity during pregnancy was controversial, as the heat produced during exercise was considered as a possible fetal teratogen (21). However, data from at least seven observational epidemiological studies provided evidence that increased physical activity before and/or during pregnancy were related to a lower risk of GDM (21). In a systematic review and meta-analysis of five studies, including 361 GDM cases, recreational physical activity in early pregnancy was related to a >20% reduction in the risk of GDM (22). Similarly, in a meta-analysis of seven studies, including 34,929 women, pre-pregnancy physical activity was on average related to a reduction in GDM risk by >50% (22). Among women who did not engage in vigorous activities, a brisk walking pace and stair climbing also were associated with a lower risk (23).

Dietary factors are also of vital importance as far as the risk of GDM is considered. Potential harmful diet patterns include the western type, characterized by high consumption of fried food, red meat, refined grain products, nutrients and beverages with high sugar content (24,25). On the other hand, potential beneficial factors include a diet rich in fresh fruits and vegetables, fish, poultry, nuts and fiber content, constituting the Mediterranean pattern (24, 26, 27). There is evidence that the latter could lower the risk of GDM (28) .

Some micronutrients seem also to contribute in GDM development and progress. One of them, is vitamin D, which has been thoroughly studied. Current evidence suggests that maternal vitamin D insufficiency is associated with a greater risk of GDM (29). In a systematic review that studied the trials conducted in the sunny Mediterranean region concluded that despite high levels of sunshine, maternal hypovitaminosis D during pregnancy is prevalent in this region (30). Findings from the investigation of joint effects of diet and physical activity, cigarette smoking and BMI indicated that more than 45% of GDM cases might have been prevented if women adopted an overall healthy diet and lifestyle and maintained a healthy body weight before pregnancy (31) .Even moderate changes in pre-pregnancy weight can affect the risk of

GDM among obese women. This may offer further motivation for interventions aimed at reducing obesity among women of reproductive age (32).

3.1.5 Complications for mother and fetus

Women with GDM are more susceptible to some pregnancy complications such as pre-eclampsia, polyhydramnios and increased risk of caesarian section (33). What is of outmost importance, is the increased risk of T2DM later in life (34,35). The complications for the fetus and neonate are also deleterious. Macrosomia, preterm delivery, risk of caesarian section delivery, stillbirth, shoulder dystocia, neonatal hypoglycemia, hyperbilirubinemia and hypocalcemia, polycythemia and respiratory distress syndrome are the most frequent and serious complications for the fetus, when a pregnancy is complicated by GDM (17,36).

3.1.6 Diagnosis and screening

GDM carries risks for the mother and the neonate, but not all adverse outcomes are of equal clinical importance. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study, a large-scale (25,000 pregnant women) multinational cohort study, demonstrated that the risk of adverse maternal, fetal, and neonatal outcomes continuously increased as a result of maternal glycemia at 24–28 weeks, even within ranges previously considered normal for pregnancy (37). These findings have led to reconsideration of the diagnostic criteria for GDM. Thus, GDM diagnosis can be accomplished with either of two strategies:

1. “One-step” 75-g OGTT or
2. “Two-step” approach with a 50-g (non-fasting) screen followed by a 100-g OGTT for those who screen positive.

The following table can clarify the two diagnostic procedures and criteria (38) :

Table 1. Diagnostic procedures and criteria for GDM

One-step strategy:

Perform a 75-g-OGTT, with plasma glucose measurement when the patient is fasting and at one and two hours, at 24-28 week of gestation in women not previously diagnosed with overt diabetes. The OGTT should be performed in the morning after an overnight fast of at least 8 hours. The diagnosis of GDM is made when any of the following plasma glucose values are met or exceeded:

-Fasting: 92mg/dL (5.1 mmol/L)

-1 hour: 180mg/dL (10.0 mmol/L)

-2 hour: 153mg/dL (8.5 mmol/L)

Two-step strategy:

Step 1: Perform a 50-g- GLT (glucose tolerance test), with plasma glucose measurement at one hour, at 24-28 week of gestation in women with not previously diagnosed with overt diabetes. If the plasma glucose level measured 1hour after the load is 140mg/dL (7.8mmol/L) proceed to 100-g-OGTT.

Step 2: The 100-g-OGTT should be performed when the patient is fasting. The diagnosis of GDM is made if at least two of the following four plasma glucose levels (measured fasting and one hour, two hours, three hours during OGTT) are met or exceeded:

	Carpenter and Coustan (39)	NDDG (National Diabetes Data Group) (40)
Fasting	95mg/dL (5.3mmol/L)	105mg/dL(5.8mmol/L)
1hour	180mg/dL(10.0mmol/L)	190mg/dL(10.6mmolL)
2hours	155mg/dL(8.6mmol/L)	165mg/dL(9.2mmol/L)
3hours	140mg/dL(7.8mmol/L)	145mg/dL(8.0mmol/L)

A cost-benefit estimation comparing the two strategies concluded that the one -step approach is cost-effective only if patients with GDM receive post-delivery counseling and care to prevent T2DM (41).

As the IADPSG (International Association of the Diabetes and Pregnancy Study Groups) criteria ("one-step strategy") have been adopted internationally, further evidence has emerged to support improved pregnancy outcomes with cost savings (42) and was the preferred approach until recently.

There are certain factors that place women at a lower risk for the development of glucose intolerance during pregnancy, and it is likely not cost-effective to screen such patients. Pregnant women who fulfill all of these criteria need not to be screened for GDM according to these recommendations.

This low-risk group comprises women who:

- are <25 years of age
- are a normal body weight
- have no family history (i.e., first-degree relative) of DM
- have no history of abnormal glucose metabolism
- have no history of poor obstetric outcome
- are not members of an ethnic/racial group with a high prevalence of diabetes (e.g., Hispanic American, Native American, Asian American, African American, Pacific Islander)

Risk assessment for GDM should be considered at the first prenatal visit. Women with clinical characteristics consistent with a high risk of GDM (marked obesity, personal history of GDM, glycosuria, or a strong family history of DM) should undergo glucose testing as soon as possible. If they are found not to have GDM at the initial screening, they should be retested between 24 and 28 weeks of gestation. Women of average risk should have testing undertaken at 24 –28 weeks of gestation (38). Obese women should be treated and monitored closely, as both GDM and obesity independently contribute to adverse pregnancy outcomes and women who are obese and have GDM are clearly at highest risk (43).

However, the Endocrine society suggests universal screening (44). More specifically, it is suggested that a fasting plasma glucose, HbA1C, or an untimed random plasma glucose should be tested at the first prenatal visit (before 13 weeks gestation or as soon as possible thereafter) for those women not known to already have diabetes. In the case of overt diabetes, but not gestational diabetes, a second test (either a fasting plasma glucose, untimed random plasma glucose, HbA1C, or OGTT) must be performed in the absence of symptoms of hyperglycemia and found to be abnormal on another day to confirm the diagnosis. The Endocrine society criteria are summarized at the following table:

Table 2: Diagnostic criteria for overt diabetes and GDM at the first prenatal visit (before 13 weeks gestation or as soon as possible thereafter) for those women not known to already have diabetes.			
Diagnosis	**Fasting Plasma Glucose, mg/dL (mmol/L)**	**Untimed (Random) Plasma Glucose, mg/dL (mmol/L) [1]**	**HbA1C, %**
Overt diabetes (type 1, type 2, or other)	≥126 (≥7.0)	≥200 (≥11.1)	≥6.5%
Gestational diabetes	92–125 (5.1–6.9)	NA	NA

Abbreviation: NA, not applicable.

1: Testing should use plasma glucose analyzed at a laboratory, not capillary blood glucose analyzed with a blood glucose meter.

3.1.7 Prevalence

Late pregnancy is characterized by a two-third decrease in insulin sensitivity, thus in most cases the first recognition of women with glucose intolerance occurs during pregnancy (45). Reported frequencies of GDM vary widely among the world. When rates of GDM were calculated in individual HAPO collaborating centers using IADPSG criteria, rates differed substantially, range 9–26% (46). HAPO Study participants represent consenting research study volunteers and frequencies of GDM that were found in specific centers may not be representative of national or regional population-based data (41). In the USA, the percentage of women affected by GDM reaches 10%, but in some countries such as India or China the prevalence is almost 20% (45-49). In European women GDM develops in 2%- 6% of pregnancies. In 90% of these women nutritional counseling is sufficient but, in the remainder, pharmacologic therapy is required (50). In the USA, there is an increasing trend in GDM, rising from 1.9% during the years 1989-1990 to 4.2% in 2003-2004 (51,52).

3.1.8 Risk of recurrence

There is evidence that the hyperglycemic intra-uterine environment has an enduring effect on the off-spring, adding to the genetic background the risk of developing obesity, cardiovascular and other metabolic diseases, constituting a serious public health issue. The off-spring is in greater risk to become obese, and by the time of child-bearing age the probability of developing GDM is considerably greater (53). It appears that an elevated fasting serum glucose that is revealed during pregnancy, is a marker of underlying metabolic abnormalities that may have preceded pregnancy (54) and may predict a higher likelihood of T2DM later in life (55).

There are a number of studies that examine the recurrence of GDM in future pregnancies. Gaudier et al. found a 52% increased risk of recurrence of GDM in subsequent pregnancies (56). In another study, Moses noticed a 35% recurrence rate after following-up 100 women (57). A cohort study in California was performed during 1991 to 2008, including 540.956 women. The rate of GDM in second and third pregnancies in women without a history of GDM was noted to be 42 and 47 per 1000 births, respectively (58). As compared with women without previous GDM in their first pregnancies, women with a first pregnancy complicated by GDM were at significantly increased risk (OR, 13.2; 95% CI, 12.0–14.6) of developing GDM during their second pregnancy. As compared with women without GDM in their first and second pregnancies, women with pregnancies complicated by GDM during their first but not second pregnancies were found to be at 6.3-fold (95% CI, 4.5–9.0) increased risk for developing GDM during their third pregnancy. This risk was even more pronounced in the third pregnancy for women who developed GDM during their first and second pregnancies (OR: 25.9; 95% CI, 17.4–38.4). In this retrospective cohort study with a large number of participants it was shown that the risk of GDM is significantly increased in the third pregnancy if the first pregnancy was not complicated by GDM and the second pregnancy was with GDM, as compared to if the first pregnancy was with GDM and the second without. It was also noticed that the risk of recurrence was higher if both pregnancies were complicated by GDM (58).

3.2Vitamin B12

3.2.1 Vitamin B12 structure

Vitamin B12 or cobalamin is a water-soluble vitamin that is implicated in various metabolic procedures, hence is interesting to study its function. B12 consists of a central cobalt atom surrounded by a heme-like planar corrin ring structure, with the four pyrrole nitrogens coordinated to the cobalt (59). Naturally it occurs in the form of 50-deoxyadenosylcobalamin (coenzyme B12) and methylcobalamin (MeCbl) (60). The structure of B12 is shown at the following figure:

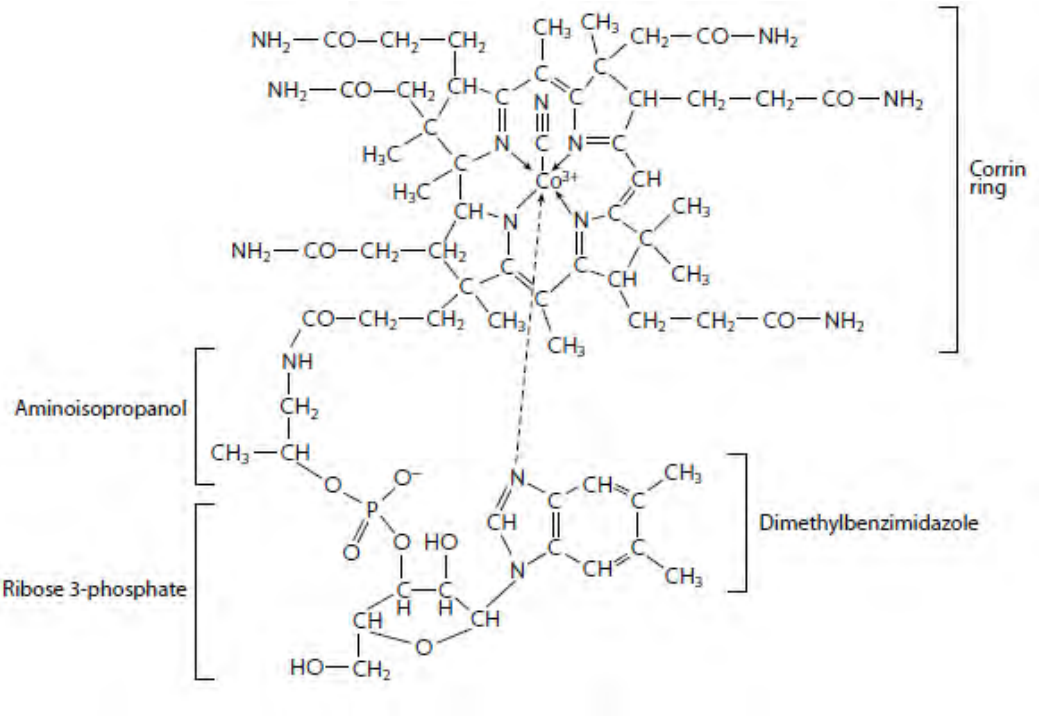

Figure 2: The structure of vitamin B12 (59)

3.2.2 Absorption-transport-bioavailability

The bioavailability of vitamin B12 in foods, depends on its amount in the diet, but in general terms is around 50% (61). Nutrient bioavailability broadly refers to the proportion of a nutrient that is absorbed from the diet and used for normal body functions. In food, vitamin B12 is bound to protein (such as albumin)

and is released in stomach by the action of pepsin in the acid environment, during a process named ‘proteolysis’. The released B12 binds initially to R-binders which are dietary proteins that have affinity for B12. The stomach epithelium contains the parietal cells that secrete gastric acid (by the ATPase) and a glycoprotein of 50 kDa called ‘intrinsic factor’ (IF) that binds to vitamin B12. As the vitamin B12-R binder complexes pass through the small intestine, the R-binders are hydrolyzed by pancreatic proteases and the free vitamin B12 binds to IF. Following a meal, sufficient IF is secreted, in order to bind 2-4 μg of B12. The vitamin is absorbed at the distal ileum via receptor mediated endocytosis. The IF-receptor (cubulin) recognizes the IF-vitamin B12 complex, it is internalized by an endocytosis- mediated procedure (62). The endosomes then fuse with lysosomes; the IF is degraded and the vitamin B12 is released in the cytosol. Vitamin B12 is released from the gut epithelial cells as a complex bound to a 38 kDa protein called ‘transcobalamin II’ (TC-II). This absorption process has a duration about 3-4 hours (63). Vitamin B12 can be also absorbed by a diffusion-like process, but it is not very efficient as less than 1% of the provided B12 is absorbed. This process however, could be important for individuals who lack IF or malabsorb vitamin B12 from food. The TC-II complex carries absorbed vitamin B12 to the tissues. The half-life of this complex is about 6 minutes. The main organ that stores vitamin B12 is the liver, in a quantity of 2-3mg in the form of 5-deoxyadenosylcobalamin attached to methylmalonylCoA mutase, whereas in plasma the major form of B12 is methylcobalamin. The excretion of vitamin B12 is via the bile and urine. The enterohepatic circulation results in the effective reuptake. The turnover rate is estimated at about 0,1% per day (64).

3.2.3 Recommended dietary intake-prevalence of B12 deficiency

The amount of B12 that is stored in the liver may be sufficient without repletion for 3-5 years. The efficient enterohepatic turnover ensures minimal losses of B12. Generally, more than 2.4μg/day are considered as adequate intake. As the liver stores of B12 are depleted, TC-II concentrations start to fall before any decline of the total serum vitamin B12 level is observed. Therefore, TC-II levels are considered more sensitive for detecting B12 insufficiency (65). Moreover, biomarkers of B12 insufficiency are elevated serum concentrations of methylmalonic acid (MMA) and homocysteine. Elevated homocysteine is a non-specific biomarker of B12 insufficiency since it is influenced by folate concentration as well. On the other hand, plasma MMA is specific for B12 status (66).

The prevalence of vitamin B12 deficiency in UK and the USA is 6% and 20% in population ≤60 years and ≥60 years respectively (67). B12 deficiency is far more prevalent in Asia, and Africa. In India for instance, 80% of pre-school children and 70% of adults are vitamin B12 deficient (67).

3.2.4 Biochemical function

Vitamin B12 has a crucial role in the methylation of DNA, and cell metabolism, as activated coenzyme B12 acts as methyl donor for the methylation processes of DNA and RNA. Vitamin B12 deficiency signifies that there would be a disruption of DNA synthesis and cell cycle, homocysteine levels increase, and cell division and differentiation would be negatively affected (68). Vitamin B12 exerts its physiological effects through mediating two principal enzymatic pathways: the methylation process of homocysteine to methionine and the conversion of methyl malonyl coenzyme A (CoA) to succinyl-CoA. Vitamin B12 as a co-factor is implicated in the process of methylation of homocysteine to methionine, which is later activated into S-adenosyl-methionine that donates its methyl group to methyl acceptors such as myelin, neurotransmitters and membrane phospholipids (68). As it was mentioned before, vitamin B12 deficiency could lead to hyperhomocysteinemia, which can have toxic effects on the neurons and the vascular epithelium. Other complications of B12 deficiency are impaired synthesis of neurotransmitters like serotonin and dopamine, and defective fatty acid synthesis (69).

3.2.5 Vitamin B12 and diabetes mellitus

Several cross-sectional studies (70-72) and case reports (73-75) have studied vitamin B12 status among patients with T2DM. In those studies, metformin was associated with B12 deficiency. However, less is known about the association of vitamin B12 and the risk of GDM.

3.3 Purpose of the present review

Vitamin B12 is a micronutrient that has many important biochemical functions and is implicated in many metabolic pathways. It is a vitamin that is studied in a lesser extent as far as its association with metabolic diseases is concerned. Therefore, it is very interesting to study its role and the consequences of its deficiency in the context of GDM that is a metabolic disease affecting a percentage of pregnant women that is increasing. Therefore, the primary aim of this study was to systematically investigate and meta-analyze the evidence for the association between B12 deficiency and the risk of GDM. We also tried to elucidate the contributory role of potential confounders, such as body mass index, homocysteine, folate and triglyceride concentrations, in this association.

3.4 Materials and methods

3.4.1 Search strategy

A comprehensive search was conducted up to November 30th, 2018. We researched relevant studies in PubMed (Medline), Cochrane Central Register of Controlled Trials and Scopus. The following search string was used in PubMed: ((((((((((gestational diabetes[MesH]) OR pregnancy diabetes[tiab]) OR gestational hyperglycaemia[tiab]) OR gestational hyperglycemia[tiab]) OR hyperglycaemia pregnancy[tiab]) OR hyperglycemia pregnancy[tiab])) OR glucose pregnancy[tiab])) AND ((vitamin B12[MeSH]) OR hydroxocobalamine[tiab]))).

For Scopus the following search string was used: ((((((((((gestational AND diabetes[title/abstract]) OR pregnancy AND diabetes[title/abstract]) OR gestational AND hyperglycaemia[title/abstract]) OR gestational AND hyperglycemia[title/abstract]) OR hyperglycaemia AND pregnancy[title/abstract]) OR hyperglycemia AND pregnancy[title/abstr act])) OR glucose AND pregnancy[title/abstract])) AND ((vitamin AND b12[title/abstract]) OR hydroxocobalamine[title/abstract])))

3.4.2 Selection of studies

Inclusion criteria were: (i) Studies providing data about the vitamin B12 concentrations during pregnancy and the development of GDM; (ii) Studies providing extractable data; (iii) Studies providing exact number of pregnant women with GDM and vitamin B12 sufficiency and deficiency. Both cohort and case-control studies were eligible. Studies were excluded if: (i) They were referring to other vitamins apart from B12, not directly linked to GDM; (ii) They were conducted in vitro; (iii) The control group (women with non-GDM) was missing; (iv) They were written in non-English language; (v)They included women on antidiabetic medication or were diagnosed with DM before pregnancy. Exclusion criteria and studies excluded with reasons are available as supplementary tables 1 and 2.

3.4.3 Data extraction

The following data were recorded: (i) First author; (ii) Year of publication; (iii) Country in which the study was conducted; (iv) Study design (case control/cohort); (v) Duration of the study; (vi) Criteria of definition of GDM; (vii) Total number of pregnant women that participated in the study; (viii) Number of women with GDM; (ix) Ethnicity; (x) Trimester of pregnancy that the study was conducted. Parameters such as the age and BMI of women with and without GDM, as well as vitamin B12, folate, homocysteine and triglyceride concentrations were recorded for both groups (GDM and non-GDM women).

3.4.4 Risk of bias and study quality assessment

Newcastle-Ottawa scale (NOS) was used for assessing the quality of each study. This system evaluates studies based on three criteria: (i) Participant selection; (ii) Comparability of study groups; (iii) Assessment of the outcome or exposure. A study can be awarded a maximum of four stars for the selection category, a maximum of two stars for the comparability category and a maximum of three stars for the outcome/ exposure category. These results are available in Supplementary table 3.

3.4.5 Statistical analysis

The following comparisons were made according to the incidence or prevalence of GDM: (i) Pregnant women with GDM and vitamin B12 sufficiency; (ii) Pregnant women with GDM and vitamin B12 deficiency; (iii) Pregnant women with normal glucose homeostasis and vitamin B12 sufficiency; (iv) Pregnant women with normal glucose homeostasis and vitamin B12 deficiency. Additionally, vitamin B12 concentrations in GDM and non-GDM women were compared. Heterogeneity was tested with the Cochrane chi-square test and the degree of heterogeneity was quantified by the I^2 statistics. An I^2 of 30-60% was considered as moderate, whereas values >60% were considered as high degree of heterogeneity. Associations were reported as odds ratios (OR) with their 95% confidence intervals (CI). A p value of <0.05 was considered as statistically significant. All analyses were performed with the latest version of *STATA* software.

3.5 Results

3.5.1 Descriptive data

Initial screening yielded 122 publications. After removing duplicates, 97 publications were assessed for eligibility. Of those, 87 were excluded based on their title and/or abstract information. Ten studies were assessed as full texts for eligibility, four of those were excluded due to the following reasons: (i) The study was conducted in vitro (n=2); (ii) The study did not provide data on women with GDM (n=1); (iii) The study was written in non-English language (n=1). The reasons for exclusion are summarized in the supplementary table 2. Six studies were included in the qualitative analysis and two studies were included in the quantitative analysis (76-81), two of which (76,77), could be meta-analyzed. The rest four studies (78-81) are excluded because no data on the exact number of GDM according to B12 deficiency/sufficiency are provided. A flow diagram of the systematic review is presented below:

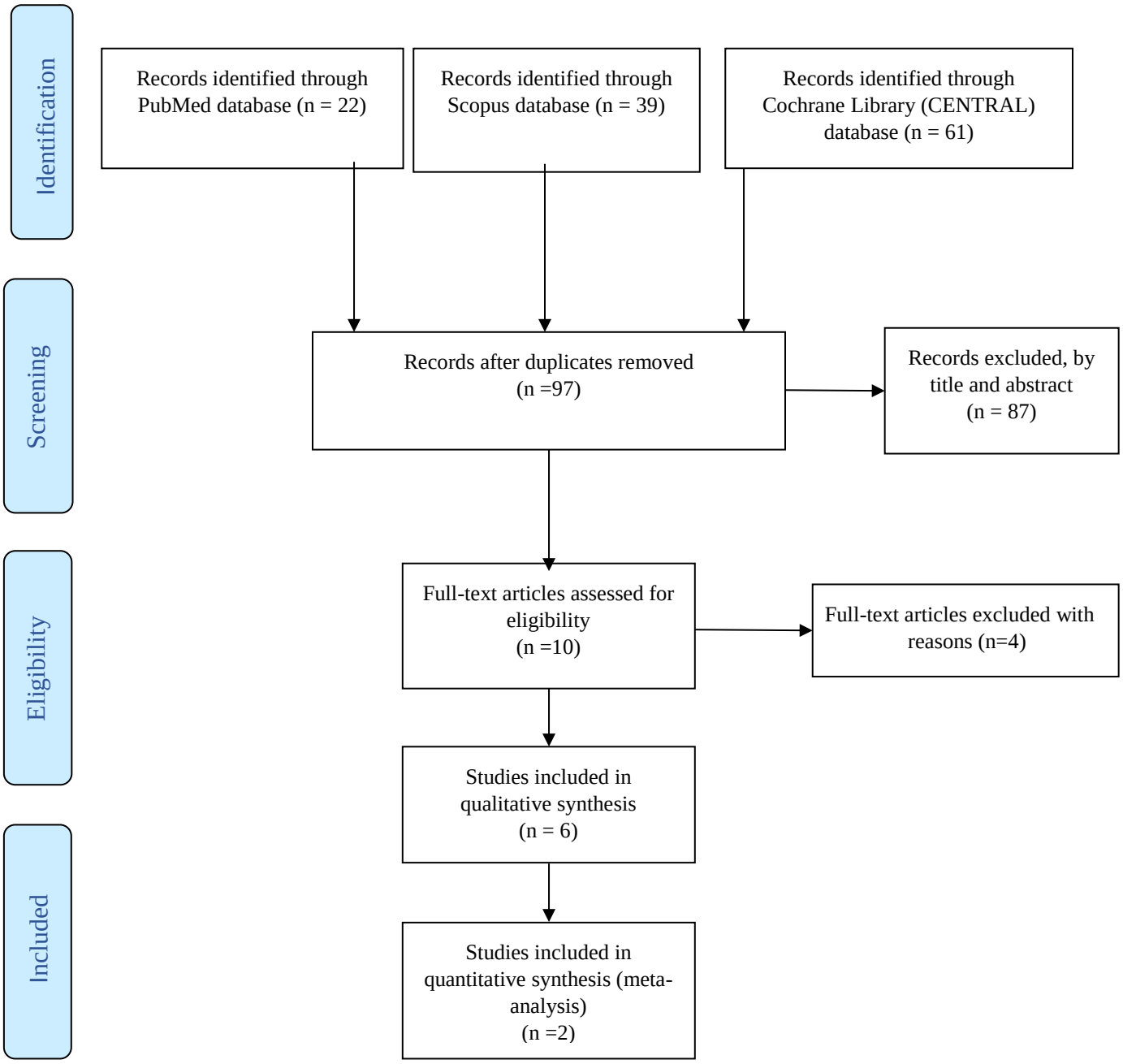

Figure 3: Flow chart diagram

All studies were published between 2003 and 2016. The countries in which they were conducted were: UK (n=1), Turkey (n=2), India (n=1), Italy (n=1), Poland (n=1). The studies were of prospective (n=2), case-control (n=2) and cross-sectional (n=2) design. The number of participants in the studies ranged from 61 to 785 pregnant women, yielding a total number of 1810, with 309 cases of GDM. The duration of studies varied from one semester to three years (data available from 6 studies). The criteria for diagnosis of GDM that were mostly used were Carpenter and Coustan (82), but in some studies the WHO 1999 (5) or the American Diabetes Association criteria (83) were used. Apart from indigenous pregnant populations, cohorts from UK (76) included pregnant women of different origin, with different social and cultural/nutritional habits.

Cohorts from Turkey (78,79) included the most homogenous native populations. The studies' descriprive characteristics are summarized in the table 3. Table 4 summarizes the laboratory methods that were used in order to measure glucose and definition of vitamin B12 deficiency.

Table 3. Study characteristics

ID	Author	Year of publication	Country	Study design	Study duration (years)	GDM criteria	Number of pregnant women	Number of women with GDM	Ethnicity	Trimester of pregnancy
1	Sukumar	2016	UK	Case-control	3	WHO 1999	344	143 (41.6%)	European: 86.9% S.Asian: 9.3% Afro-Caribbean: 1.2% Other: 1.2%	3rd
2	Krishnaveni	2009	India	Prospective	2	Carpenter and Coustan	785	49 (6.2%)	Hindu: 59.5% Muslim: 33.1% Other: 3.8	3rd
3	Tarim	2004	Turkey	Prospective	1	Carpenter and Coustan	304	28 (9.2%)	Turkish	3rd
4	Guven	2006	Turkey	Cross-sectional	2	Carpenter and Coustan	223	30 (13.4%)	Turkish	3rd
5	Seghieri	2003	Italy	Cross-sectional	one semester	American Diabetes Association	93	15 (16.1%)	Italian	3rd
6	Idzior-Walus	2008	Poland	Case-control	No data	WHO 1999	61	44 (72.1%)	No data	3rd

Table 4. Vitamin B12 assay characteristics and status definition						
ID	First author	Year	Method of blood glucose assessment	Method of serum vitamin B12 assessment	Vitamin B12 status definition (pmol/L)	
					Definition	Range
1	Sukumar	2010-2013	Hexokinase enzymatic method	Electrochemiluminescent immunoassay	<150pmol/L	150-489pmol/L
2	Krishnaveni	1997-1998	Microbiological assays	Microbiological assays	<150pmol/L	Not defined
3	Tarim	2002-2003	Glucose oxidase method	Electrochemiluminescence technique	Not defined	Not defined
4	Guven	2002-2004	Glucose oxidase method	Automatic chemiluminescent method	Not defined	Not defined
5	Seghieri	2001	Glucose GOD-PAP	Chemiluminescent Immunoassay	Not defined	Not defined
6	Idzior-Waluś	2008	Immunoradiometric method	Chemiluminescent immunoassay	Not defined	Not defined

Abbreviations: GOD-PAP: glucose oxidase-phenol and 4 aminophenazone

3.5.2 Comparison of GDM with non-GDM women based on the age and BMI

Very useful information is provided the following table (Table 5), analyzing the characteristics of the pregnant women that participated in the studies. The age and Body Mass Index (BMI) of women with normal glucose tolerance and those of women with gestational diabetes mellitus is compared.

Table 5: Characteristics of the participants according to age and body mass index (BMI)									
ID	First author	Mean age of of the entire cohort (years)	Mean age of GDM women (years)	Mean age of non-GDM women (year)	P-value[1]	Mean BMI of the entire cohort ($kg/m^{2)}$	Mean BMI of GDM women ($kg/m^{2)}$	Mean BMI of non-GDM women ($kg/m^{2)}$	P-value[2]
1	Sukumar	30.3 ±5.88	31.4 ±5.8	29.6 ±5.9	P=0.0053	28.8 ±7.46	31.7 ±7.0	26.7 ±7.1	P<0.0001
2	Krishnaveni	23	N/A	N/A	-	23.1	N/A	N/A	-
3	Tarim	N/A	32 ±4.03	26.83 ±4.44	P<0.0001	N/A	27.12 ±2.1	25.24 ±1.99	P<0.0001
4	Guven	N/A	30.0 ±4.3	28.6 ±3.4	P=0.0446	N/A	29.17 ±4.11	27.88 ±2.80	P=0.0297
5	Seghieri	N/A	34.6 ±3.1	32.3 ±3.7	P=0.0264	N/A	26.7 ±3.2	26.3 ±3.7	P=0.6966
6	Idzior-Walus	N/A	30.5 ±6.6	26.2 ±4	P=0.0150	N/A	27.8 ±5.2	25.6 ±3.4	P=0.1123

Data are presented as mean ± SD. Abbreviations: BMI: Body Mass Index; GDM: Gestational Diabetes Mellitus; N/A: Not Available

P-value[1:] comparison of the age between the groups of GDM and non-GDM women

P-value[2:] comparison of the BMI between the groups of GDM and non-GDM women

All but one (Krishnaveni et al.) studies, provided information about the and BMI of the included. As expected, women with GDM, tended to be older and with higher BMI when compared with women with normal glucose homeostasis.

3.5.3 Comparison of vitamin B12 concentrations between women with GDM and women with and without GDM

We obtained data from the six studies with regard vitamin B12 concentrations in both groups of pregnant women (GDM and non-GDM) (Table 6 and Table 7).

Table 6. Association between maternal vitamin B12 concentrations and GDM					
ID	First author	Vit.B12 deficiency		GDM/vit. B12 deficiency	GDM/ vit. B12 sufficiency
		YES	NO		
1	Sukumar	90(26.2%)	254(73.8)	46(51.1%)	97(38.2%)
2	Krishnaveni	334 (43.2%)	439 (56.7)	29(8.7%)	20 (4.6%)
3	Tarim	N/A	N/A	N/A	N/A
4	Guven	N/A	N/A	N/A	N/A
5	Seghieri	N/A	N/A	N/A	N/A
6	Idzior-Walus	N/A	N/A	N/A	N/A

Data are presented as mean ± SD or median

Abbreviations: Vit.: Vitamin; N/A: Not Available

Table 7. Comparison of B12 concentrations between GDM and non-GDM women				
ID	First author	Vitamin B12 concentrations (pg/mL)		P value[1]
		Normal glucose tolerance	GDM	
1	Sukumar	195.6(157.9-244.6)	169 (140.2-217.7)	P<0.01
2	Krishnaveni	N/A	N/A	N/A
3	Tarim	161.8±55.3	150.8±45.5	P=0.1066
4	Guven	234.5± 295.9	160.4±31.1	P=0.0008
5	Seghieri	229.93±44.32	242.97±39.24	P=0.2513
6	Idzior-Walus	287±37.5	262± 82.6	P=0.1079

P-value1: comparison of the groups GDM and non-GDM women for B12 concentrations

Data are presented as mean ± SD or median

Abbreviations: N/A: Not Available

Apart from one study by Krishnaveni et al. (77), the rest five studies provided data on vitamin B12 concentrations for both GDM and non-GDM groups. Women with GDM had lower levels of vitamin B12 when compared with non-GDM women. Moreover, a significantly higher proportion of women with GDM had B12 insufficiency compared with non-GDM in all the eligible studies.

3.5.4 Comparison between folate, homocysteine, and triglyceride concentrations between GDM and non-GDM women

Table 8 presents data on folate, homocysteine and triglyceride concentrations, for both groups of pregnant women, GDM and non-GDM.

Table 8. Folate, Homocysteine and Triglyceride concentrations in GDM and non-GDM women

ID	First author	Folate non-GDM women (nmol/L)	Folate GDM women (nmol/L)	Homocycteine non-GDM women (μmol/L)	Homocycteine GDM women (μmol/L)	Triglycerides non-GDM women (mg/dL)	Triglycerides GDM women (mg/dL)
1	Sukumar	20.8 (14.5-34.4)	21.5 (13.5-34.5)	N/A	N/A	N/A	N/A
2	Krishnaveni	N/A	N/A	N/A	N/A	N/A	N/A
3	Tarim	25.1±10.9	14.36±5.05	4.80±0.98	5.70±0.9	180.06±40.95	249±43.53
4	Guven	15.1±7.25	14.36±5.05	7.4±1.6	9.0±3.1	N/A	N/A
5	Seghieri	31.3±16.5	33.3±17.9	4.45±1.52	5.88±2.26	212.3±70.79	247.7±115.04
6	Idzior-Walu	25.1±13.3	25.3±13.59	7.4±1.1	8±2	168±44.24	238±79.64

Data are presented as mean ± SD or median

Abbreviations: N/A: Not Available

It is observed that the levels of folate are almost the same in GDM and non-GDM women. This fact may be attributed to the folate supplements that most women are given by their medical practitioner since the very beginning of their pregnancy (76).

As far as homocysteine is concerned, we can observe that the levels are increased in the group of pregnant women with GDM when compared to the group of women with normal glucose tolerance, as well as the triglyceride concentrations.

For the homocysteine levels, Tarim et al. observed a p value of $p<0,001$, Guven et al. a p value of $p<0,01$, Seghieri et al. a p value of $p=0,003$ and finally Idzior-Walus et al. a statistically non-significant p value, when the groups of women with normal glucose tolerance and women with GDM were compared.

When comparing the results obtained for the triglyceride levels, the study of Tarim et al. observed a p value $p<0,0001$, the study of Idzior-Walus et al. a p value $p=0,002$ and finally Seghieri et al. a p value that was statistically non-significant.

3.5.5 Meta-analysis

Only two of the six studies included in this systematic review could be meta-analyzed (Sukumar et al, Krishnaveni et al.) (76,77). The rest of studies did not provide data on the exact number of pregnant women with GDM according to vitamin B12 status.

Pregnant women with vitamin B12 deficiency demonstrated an increased risk of developing GDM compared with those with vitamin B12 deficiency: OR 1.81 (95% CI 1.25-2.63), with very low heterogeneity (Figures 4 and 5).

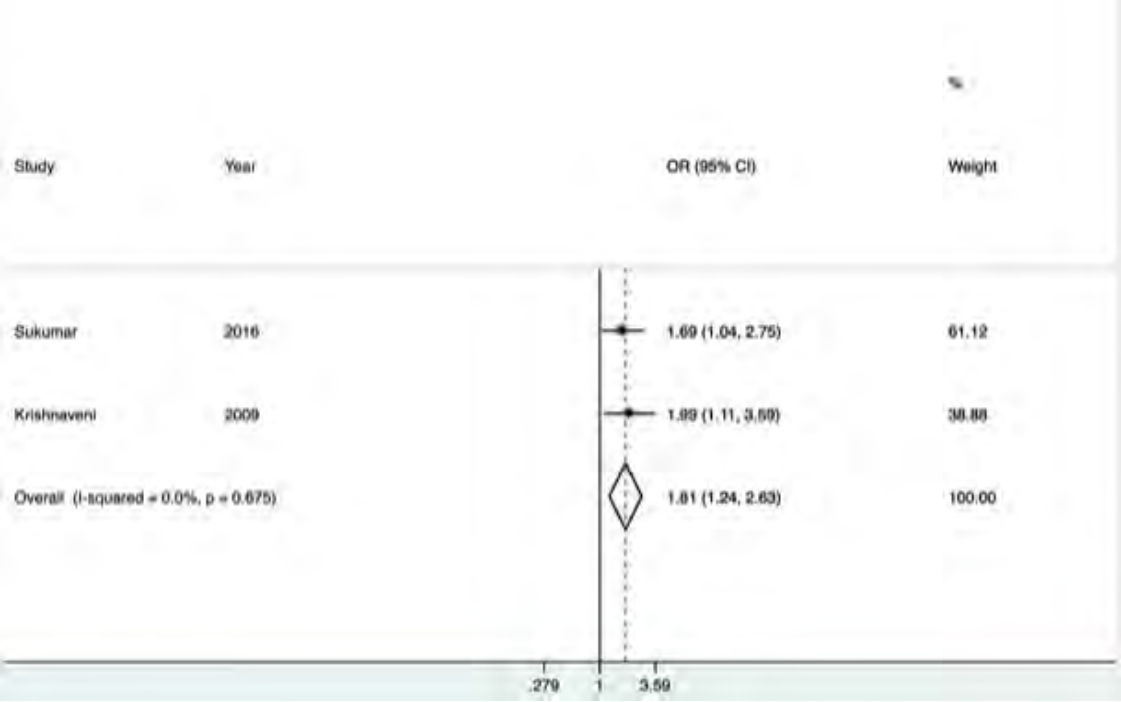

Figure 4: Meta-analysis results

Study	OR	[95% Conf. Interval]		% Weight
Sukumar	1.692	1.042	2.747	61.12
Krishnaveni	1.992	1.106	3.588	38.88
M-H pooled OR	1.809	1.245	2.628	100.00

Heterogeneity chi-squared = 0.18 (d.f. = 1) p = 0.675
I-squared (variation in OR attributable to heterogeneity) = 0.0%
Test of OR=1: z= 3.11 p = 0.002

Abbreviations: OR: Odds Ratio; Conf.Interval: Confidence Interval
d.f: degrees of freedom; M-H: Mantel-Haenszel

Figure 5: Statistics results of the meta-analysis

3.6 Discussion

GDM is the most common metabolic disorder during pregnancy and is also associated with adverse effects on pregnancy, on the vascular system and is also associated with birth defects (84,85). In a systematic review and meta-analysis by Eades et al., published in 2017, that included 40 studies conducted in 11 European countries with a total population of 1.778.399 participants, the mean prevalence of GDM was 5.4% (95% CI, 3.8-7.8%). The highest prevalence was found in studies conducted in Italy (10%; 95% CI, 7.6- 13) and the lowest in Sweden (1.5%;95% CI, 1-2.3). Highest prevalence was found in countries in Southern Europe (9.6%; 95% CI, 7.3-12.6) and lowest in Northern Europe (2.3%; 95% CI, 1.3- 3.8) (86). Another important finding of this meta-analysis was that the prevalence of GDM increased every decade, with the lowest in the 1980's (0.9%, range: 0.1-10) and the highest in the 2010's (11.1%, range: 5.7-20.6).

This increasing prevalence of GDM and the harmful consequences for both the pregnant woman and the newborn, make the study of this metabolic disease interesting and the researchers are provoked to elucidate the possible pathophysiological mechanisms and the relation of the disease to variable modifiable parameters.

Vitamin B12 seems to have a fundamental role in the synthesis of DNA, proteins and lipids. Along with folate, they participate in a series of cellular reactions collectively known as one-carbon metabolism, as essential cofactors for the synthesis of methionine from homocysteine (87) and at the mitochondrial level, vitamin B12 is required as a coenzyme for the conversion of methylmalonyl-CoA to succinyl-CoA, in the process of fatty acid oxidation. In the absence of vitamin B12 the process is inhibited and lipogenesis is promoted (88).

To the best of our knowledge, this is the first systematic review and meta-analysis on the association between vitamin B12 deficiency and GDM. Regarding the primary endpoint, we found two studies providing available data. It was shown that pregnant women who are vitamin B12 deficient are almost at two-fold increased risk of developing GDM. With regard to the prevalence of B12 deficiency, in the first study conducted in UK (76), 26.2% of pregnant women were B12 deficient, while in the second study, conducted in India (77) where vegetarianism is part of the country's culture, almost half of the population was deficient (43.2%).

Regarding the second endpoint, in an attempt to elucidate potential parameters that could explain this phenomenon of discrepancy of the percentages of vitamin B12 deficient and non-deficient pregnant women, we found differences in folate, homocysteine and triglyceride concentrations as well as in BMI regarding B12 deficiency or sufficiency in pregnant women.

Folate deficiency is rare during pregnancy due to the folate supplements that women take since the very beginning of their pregnancy (91% of the cohort was taking folate supplements in the study by Sukumar et al.) (76). In the same study, the serum folate levels did not have a significant difference in the group of women with GDM and non-GDM women. However, after adjusting for age, parity, ethnicity, smoking status, serum folate showed a positive association with vitamin B12 (β coefficient :0.23; p-value <0.001). Folate deficiency in this study was not significantly associated with a risk of GDM. In the study by Seghieri et al. (80) that studied folate concentrations during pregnancy, there was not a remarkable difference between the concentrations in the two groups of pregnant women, folate concentrations for non-GDM and GDM group were: 31.3 ± 16.5 and 33.3±17.9 nmol/L respectively. The p-value was not significant.

As far as homocysteine is concerned, hyperhomocysteinemia, as a marker of both folate and vitamin B12 deficiency, has been studied and identified as a risk factor for cardio-vascular diseases and insulin resistance-DM (89-91). Four studies included in this systematic review, Tarim et al. (78), Guven et al. (79), Seghieri et al. (80), Idzior-Walus et al. (81), reported that homocysteine levels were increased in pregnant women with GDM when compared with non-GDM women. This increase is unlikely due to the possible differences in oral folate supplementation or in serum albumin, the two possible mechanisms that are previously identified as modulators of homocysteine concentration during pregnancy (92). Homocysteine

concentrations, can be modified and regulated by several factors, for example genetically determined enzyme alterations, nutritionals status, underlying diseases, age, medications and pregnancy. The latter is the only one that specifically decreases plasma homocysteine concentrations (92). In general, during pregnancy, homocysteine levels are lower than normal, and this is due to either a physiological response to the pregnancy, an increase in estrogen concentration, hemodilution from the increased plasma volume of the pregnant woman, or by an increased demand for the amino acid methionine from the mother and the fetus (93). Three studies, Tarim et al. (78), Guven et al. (79), Seghieri et al. (80), included in our systematic review that provided data about homocysteine concentrations in women with GDM and women with normal glucose homeostasis, concluded that they are increased in GDM women. However, Idzior-Walus et al. (81) did not observe any difference in homocysteine levels in the blood between GDM and non-GDM pregnant women. This means that more studies are needed to confirm this information. In the study by Guven et al. (79), there was a weak partial correlation, controlled for homocysteine, between serum vitamin B12 and folate levels ($r=0.22$, $p=0.01$). However, serum homocysteine levels demonstrated a negative partial correlation, when controlled for vitamin B12, with serum folate ($r= -0.172$, $p=0.02$). Idzior-Walus et al. (81), observed that in women with GDM, serum vitamin B12 concentrations correlated negatively with homocysteine ($r= -0.47$, $p<0.01$). More studies are thus needed, in order to prove the relation between vitamin B12, homocysteine and GDM.

Hypertriglyceridemia is a common feature in insulin-resistant patients (94). Triglycerides may increase oxidative stress and impair insulin action (95). Idzior-Walus et al. (81) demonstrated that in women with GDM serum vitamin B12 concentrations are correlated negatively with serum triglycerides ($r= -0.42$, $p<0.01$). Tarim et al. (78), observed that the mean triglycerides and very-low density lipoprotein (VLDL) concentrations in the group of GDM women were significantly higher than those of the control group ($p<0.05$). In the study by Seghieri et al. (80) no difference was found between the two groups. Additionally, in this study it is interesting to point-out that vitamin B12 correlated not only with total homocysteine, but also with serum triglyceride concentrations.

With respect to BMI, the results were not conclusive when pregnant women with GDM were compared with non-GDM women. Three of the studies included in this systematic review, by Sukumar et al. (76), Krishnaveni et al. (77) and Tarim et al. (78), concluded that the BMI of pregnant women with GDM was higher when compared with non-GDM women. Interestingly, Sukumar et al. (76) observed that women with vitamin B12 deficiency had higher BMI in the first trimester than those who were vitamin B12 sufficient (30.9 ± 7.56 vs. 28.0 ± 7.30 kg/m^2, $p < 0.05$). After adjusting for age, parity, ethnicity, smoking status, and gestation of blood tests, BMI was a significant negative predictor of B12 (β coefficient -0.21; 95% CI: -0.47, -0.13; $p = 0.001$). It is also impressive the fact that third trimester vitamin B12 insufficiency

was associated with 2.4 times higher risk of obesity in the first trimester. Krishnaveni et al. (77) demonstrated that vitamin B12 concentrations were adversely associated with BMI and sum of skinfolds. In the same context as Sukumar et al. (76), he observed that vitamin B12 deficiency was associated with higher BMI, larger sum of skinfolds, higher insulin resistance and increased risk of GDM. Tarim et al. (78), also showed that the BMI of pregnant women with GDM was higher than in the control group ($p<0.05$). The rest of the three studies in this systematic review by Guven et al. (79), Seghieri et al. (80) and Idzior-Walus et al. (81) did not prove any significant difference in BMI of GDM and non-GDM women.

The first study showing that women with vitamin B12 deficiency are at higher risk to develop GDM, was conducted by Krishaveni et al. (77) in India during the years 1997-1998 and published in 2009. GDM was 2.14 times more frequent (95% CI: 1.11-4.13) in vitamin B12 deficient compared with non-deficient pregnant women. The former group was also more likely to be in the highest quartile of adiposity (OR for BMI: 2.1, $p<0.001$; OR for the sum of skinfolds:1.7 $p=0.004$). In this study, both adiposity and the incidence of GDM increased as the folate concentrations increased in women with vitamin B12 deficiency. On the other hand, in women with normal vitamin B12 concentrations, insulin resistance decreased as the folate concentrations increased. However, the interaction between B12 deficiency and folate concentrations was not significant. In this study, a high prevalence of B12 deficiency was noticed (≥40%) and this was in part explained by the Indian culture and the vegetarianism as a way of lifestyle. Hindu women, who are mainly vegetarian had the lowest vitamin B12 concentrations, and whereas Muslim women, who are mainly non-vegetarian, had the lowest folate concentrations (77). The risk of GDM was higher among pregnant women with vitamin B12 deficiency and high folate concentrations. The authors reported for the first time a link between vitamin B12 deficiency, adiposity and its related disorders, proposing the term ‘diabesity’.

One possible mechanism is that B12 deficiency may promote adiposity. A second possible interpretation is that adiposity lowers plasma B12 concentrations. Especially for pregnant women, possible mechanisms include greater hemodilution, increased transfer of nutrients to the fetus or increased urinary losses due to increased glomerular filtration rate. The interesting fact is that in this study, there was a five-year follow-up of the pregnant women, and this phenomenon of ‘diabesity’ persisted even after five years after pregnancy (77). In the context of adequate or high folate concentrations, there are plausible biochemical mechanisms that resonate why vitamin B12 deficiency could cause adiposity (96). When there is deficiency of B12, folate is trapped as inactive 5-methyltetrahydrofolate (97). This leads to impaired methionine synthesis, thus impaired protein synthesis, that may hinder lean tissue deposition. In addition, impaired conversion of methylmalonyl-CoA to succinyl-CoA, for which vitamin B12 acts as a cofactor. In the context of B12 deficiency, there is accumulation of methylmalonyl-CoA, which may increase lipogenesis and insulin resistance (96). These results are in accordance with the results of the PMNS (Pune

Maternal Nutrition Study) study, also conducted in India (98). It has been suggested that the widespread vitamin B12 deficiency in India may predispose Indians to the susceptibility to adiposity and the later to DM ('diabesity') and that this phenomenon is further exacerbated by increased folate levels.

The second study that investigated the connection between maternal vitamin B12 levels during pregnancy and the risk of GDM, was published in 2016 by Sukumar et al. (76). Although this study was retrospective in design, in contrast with the previous prospective study by Krishnaveni et al. (77), confirmed its results in a UK population, for the first time. Not only this study demonstrated that low vitamin B12 status during pregnancy is associated with a higher risk for GDM, but also it was observed that higher BMI during the first semester, was an independent predictor of a B12 deficiency. An interesting remark of this study was that low levels of B12, were associated with macrosomia in the group of non-GDM women, and this could be partly mediated by maternal BMI (77).

The two studies have some differences as far as the BMI of the pregnant women, as a parameter of the link between maternal B12 deficiency and GDM, is concerned. In the study by Krishnaveni et al., although the magnitude of association of B12 deficiency and risk of GDM was the same as the second study by Sukumar et al., the significance was lost when adjusted for maternal BMI. On the other hand, the significance persisted when adjusted for maternal BMI in the second study, a fact that may be interpreted as a potential independent effect of B12.

The recent study by Knight et al. although it was not performed in a GDM population, also supports the inverse link between B12 levels and insulin resistance in pregnant Caucasian, white women (99). Prospective longitudinal studies are needed to investigate whether the presence of low B12 status in early pregnancy independently increases the risk of incident GDM and also to investigate the relation between maternal BMI and B12 status.

It is interesting to observe that the rates of vitamin B12 deficiency differ between the two studies of our meta-analysis. 26.2% in the study of Sukumar et al. (76) and 43.2% in the study by Krishnaveni et al. (77). The rates differed when we compared non-GDM populations, 21.9% and 20% respectively (76,99). It seems that vitamin B12 deficiency is not limited in Indian populations that are mainly vegetarian. Interestingly, the frying and roasting of meat products reduces the bioavailability of vitamin B12 by 20%–40% (100), so higher consumption of processed foods may increase the risk of both B12 deficiency and metabolic diseases. As it was mentioned above, a decrease in concentrations of vitamin B12 is common during pregnancy, due to a decrease in the fraction bound to the inactive haptocorrin. However, it is not clear yet if there is a decrease in the active form, holotranscobalamin (99). Another issue, is that there are not specific cut-off points for the B12 concentrations during pregnancy, and we define the deficiency according to the non-pregnant reference range, with levels <150pmol/l defined as deficiency.

In all of the studies included in this systematic review, the folate deficiency was rare, which was reassuring. This may be attributed to folate supplements that are prescribed in women since the very beginning of their pregnancy, by their healthcare practitioner. However, the combination of high folate with low vitamin B12 seem to have deleterious effects on both the mother and the newborn as it was mentioned before.

In our meta-analysis, the result was statistically significant (OR: 1.81; 95% CI, 1.25-2.63), a fact that confirms our initial hypothesis that vitamin B12 deficiency is associated with a higher risk of GDM. The present study has several limitations though. The first, is that a limited number of studies were included. Second, the diagnosis of GDM was set with different criteria. Third, not all studies investigated the association between the parameters that may affect B12 concentrations, therefore it was impossible to perform a meta-regression analysis.

More studies are needed in this context, in order to prove the direct causation of vitamin B12 deficiency with GDM, and investigate the effects of different BMI on the association between vitamin B12 and GDM, as well as the parameter age in the same concept. It will be interesting to design prospective studies in order to observe how some components of the metabolic syndrome, such as triglycerides, high-density lipoprotein (HDL) and arterial blood pressure may or not affect vitamin B12 concentrations. In addition, specific cut-off points for vitamin B12 need to be set for pregnancy, in order to avoid using the same reference range as in the non-pregnant population and be more accurate.

It is also important to mention that there are no guidelines to address how often patients with T1DM or T2DM and GDM should be supplemented with vitamin B12. The optimal supplementation dose of the vitamin is unknown as well. A study from the USA published in 2012, showed that administration of oral vitamin B12 among T2DM patients on long-term use of metformin was ineffective in terms of correcting vitamin B12 deficiency. The doses in the multivitamin formulations in this study were inadequate as it seems to correct the levels of vitamin B12 (101). There is also evidence that vitamin B complex supplementation is effective in lowering plasma homocysteine concentrations, but the mechanism seems to be unclear (102). In a multi-center, randomized, double-blind trial involving patients with diabetic neuropathy, subjects received a high-dose of vitamin B-complex supplementation or placebo. The results were inconclusive, as participants in the therapy group had significantly lower plasma homocysteine concentrations, but they also had a rapid decline in renal function and increased rates of vascular events, such as stroke and myocardial infarctions (102). Thus, no firm conclusion can be made, regarding the role of vitamin B supplementation in DM. This, stresses the need for further studies to determine the optimal vitamin B12 supplementation dose and first of all to conclude if the supplementation will be beneficial for the patients with DM.

As far as GDM is concerned, the studies about supplementation with vitamin B in general and vitamin B12 more specifically, there are scarce. There is a potential benefit from B12 supplementation during pregnancy and lactation period, as far as lactation and breast milk status is concerned (103,104). In addition, there is a benefit from vitamin B12 supplementation in early pregnancy for the subsequent neurodevelopment and growth in the off-spring (105). A study that was performed in a different population, the HOPE study (Heart Outcomes Prevention Evaluation) 2, a randomized, double-blind trial which evaluated the role of vitamin B therapy (vitamin B9: 2.5 mg, vitamin B6: 50 mg, vitamin B12:1 mg) in reducing the risk of major vascular events. The participants were aged 55 or older and had either cardiovascular disease (CVD) or DM. The conclusion was that daily vitamin B therapy reduced homocysteine concentrations by 25%, but had no beneficial effects on major CVD events except for a reduction in the incidence of stroke. (106). Thus, the role of vitamin B supplementation in patients with CVD or DM are contradictory. However, further clinical trials with larger sample sizes and longer follow-up periods are required, in order to determine the possible long-term effects and safety. The dosing of such supplements should be investigated as well, before specific vitamin supplements are widely recommended in patients with DM. Especially for the sensitive period of pregnancy, extra care should be taken when recommending any vitamin supplementation, since data are not robust. The potential harmful effects and hazards to the pregnant woman, the fetus or the newborn, that could be provoked by their administration should be studied thoroughly and special concern and attention is required from the health care practitioner, nurse, or midwife when recommending them. The recently published report 'WHO recommendations on antenatal care for a positive pregnancy experience' only recommends iron and folic acid to pregnant women (107). Maybe it is time to review these recommendations, and update the international guidelines about supplementation before and during pregnancy with vitamin B-complex.

3.7 Conclusions

In summary, this systematic review and meta-analysis in pregnant women, shows that pregnant women with vitamin B12 deficiency are at two-fold risk to develop GDM during pregnancy, when compared with those who have sufficient vitamin B12 levels. This should be taken into account when building prognostic models for detection of GDM, especially in high risk women. Well-designed prospective, cohort and interventional studies will further elucidate this association and will investigate whether supplementation with B12 before or during pregnancy could reduce this risk.

REFERENCES:

1. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine: a journal of the British Diabetic Association. 1998;15(7):539-53.
2. Landon MB, Gabbe SG. Gestational diabetes mellitus. Obstetrics and gynecology. 2011;118(6):1379-93.
3. Halperin IJ, Feig DS. The role of lifestyle interventions in the prevention of gestational diabetes. Current diabetes reports. 2014;14(1):452.
4. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. The New England journal of medicine. 2005;352(24):2477-86.
5. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: A report of WHO/IDF consultation. Geneva: World Health Organization; 2006. 2006.
6. Brody SC, Harris R, Lohr K. Screening for gestational diabetes: a summary of the evidence for the U.S. Preventive Services Task Force. Obstetrics and gynecology. 2003;101(2):380-92.
7. Ferrara A, Kahn HS, Quesenberry CP, Riley C, Hedderson MM. An increase in the incidence of gestational diabetes mellitus: Northern California, 1991-2000. Obstetrics and gynecology. 2004;103(3):526-33.
8. Gestational diabetes mellitus. Diabetes care. 2004;27 Suppl 1: S88-90.
9. Kim C. Gestational diabetes: risks, management, and treatment options. International journal of women's health. 2010; 2:339-51.
10. Heslehurst N, Rankin J, Wilkinson JR, Summerbell CD. A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989-2007. International journal of obesity (2005). 2010;34(3):420-8.
11. Kimmons JE, Blanck HM, Tohill BC, Zhang J, Khan LK. Associations between body mass index and the prevalence of low micronutrient levels among US adults. MedGenMed: Medscape general medicine. 2006;8(4):59.
12. Thurnham DI BD, Scott J, Halsted CH (2001) Water Soluble Vitamins. In: Garrow JS, James, WPT RA, editors. Human Nutrition and Dietetics. Edinburgh: Churchill Livingstone. pp. 249–88. 2001.
13. Bluestone JA, Herold K, Eisenbarth G. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature. 2010;464(7293):1293-300.
14. Standards of medical care in diabetes--2011. Diabetes care. 2011;34 Suppl 1: S11-61.

15. Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, et al. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes care. 2007;30 Suppl 2: S251-60.

16. McCance DR. Diabetes in pregnancy. Best Practice & Research Clinical Obstetrics & Gynaecology. 2015;29(5):685-99.

17. Poulakos P, Mintziori G, Tsirou E, Taousani E, Savvaki D, Harizopoulou V, et al. Comments on gestational diabetes mellitus: from pathophysiology to clinical practice. Hormones (Athens, Greece). 2015;14(3):335-44.

18. Coustan DR. Gestational diabetes mellitus. Clinical chemistry. 2013;59(9):1310-21.

19. Ben-Haroush A, Yogev Y, Hod M. Epidemiology of gestational diabetes mellitus and its association with Type 2 diabetes. Diabetic medicine: a journal of the British Diabetic Association. 2004;21(2):103-13.

20. Zhang C, Rawal S, Chong YS. Risk factors for gestational diabetes: is prevention possible? Diabetologia. 2016;59(7):1385-90.

21. Artal R, O'Toole M. Guidelines of the American College of Obstetricians and Gynecologists for exercise during pregnancy and the postpartum period. British journal of sports medicine. 2003;37(1):6-12.

22. Tobias DK, Zhang C, van Dam RM, Bowers K, Hu FB. Physical activity before and during pregnancy and risk of gestational diabetes mellitus: a meta-analysis. Diabetes care. 2011;34(1):223-9.

23. Zhang C, Solomon CG, Manson JE, Hu FB. A prospective study of pregravid physical activity and sedentary behaviors in relation to the risk for gestational diabetes mellitus. Archives of internal medicine. 2006;166(5):543-8.

24. Zhang C, Liu S, Solomon CG, Hu FB. Dietary fiber intake, dietary glycemic load, and the risk for gestational diabetes mellitus. Diabetes care. 2006;29(10):2223-30.

25. Chen L, Hu FB, Yeung E, Willett W, Zhang C. Prospective study of pre-gravid sugar-sweetened beverage consumption and the risk of gestational diabetes mellitus. Diabetes care. 2009;32(12):2236-41.

26. Bao W, Bowers K, Tobias DK, Hu FB, Zhang C. Prepregnancy dietary protein intake, major dietary protein sources, and the risk of gestational diabetes mellitus: a prospective cohort study. Diabetes care. 2013;36(7):2001-8.

27. Zhang C, Schulze MB, Solomon CG, Hu FB. A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus. Diabetologia. 2006;49(11):2604-13.

28. Tobias DK, Zhang C, Chavarro J, Bowers K, Rich-Edwards J, Rosner B, et al. Prepregnancy adherence to dietary patterns and lower risk of gestational diabetes mellitus. The American journal of clinical nutrition. 2012;96(2):289-95.

29. Lu M, Xu Y, Lv L, Zhang M. Association between vitamin D status and the risk of gestational diabetes mellitus: a meta-analysis. Archives of gynecology and obstetrics. 2016;293(5):959-66.

30. Karras S, Paschou SA, Kandaraki E, Anagnostis P, Annweiler C, Tarlatzis BC, et al. Hypovitaminosis D in pregnancy in the Mediterranean region: a systematic review. Eur J Clin Nutr. 2016;70(9):979-86.

31. Zhang C, Tobias DK, Chavarro JE, Bao W, Wang D, Ley SH, et al. Adherence to healthy lifestyle and risk of gestational diabetes mellitus: prospective cohort study. BMJ (Clinical research ed). 2014;349: g5450.

32. Glazer NL, Hendrickson AF, Schellenbaum GD, Mueller BA. Weight change and the risk of gestational diabetes in obese women. Epidemiology (Cambridge, Mass). 2004;15(6):733-7.

33. Ostlund I, Haglund B, Hanson U. Gestational diabetes and preeclampsia. European journal of obstetrics, gynecology, and reproductive biology. 2004;113(1):12-6.

34. Robitaille J, Grant AM. The genetics of gestational diabetes mellitus: evidence for relationship with type 2 diabetes mellitus. Genetics in medicine: official journal of the American College of Medical Genetics. 2008;10(4):240-50.

35. Lobner K, Knopff A, Baumgarten A, Mollenhauer U, Marienfeld S, Garrido-Franco M, et al. Predictors of postpartum diabetes in women with gestational diabetes mellitus. Diabetes. 2006;55(3):792-7.

36. McCance DR. Pregnancy and diabetes. Best practice & research Clinical endocrinology & metabolism. 2011;25(6):945-58.

37. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. The New England journal of medicine. 2008;358(19):1991-2002.

38. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes care.

39. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol.

40. National Diabetes Data Group. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 1979; 28:1039–1057982; 144:768–773018;41(Suppl 1): S13-s27.

41. Werner EF, Pettker CM, Zuckerwise L, Reel M, Funai EF, Henderson J, et al. Screening for gestational diabetes mellitus: are the criteria proposed by the international association of the Diabetes and Pregnancy Study Groups cost-effective? Diabetes care. 2012;35(3):529-35.

42. Duran A, Saenz S, Torrejon MJ, Bordiu E, Del Valle L, Galindo M, et al. Introduction of IADPSG criteria for the screening and diagnosis of gestational diabetes mellitus results in improved pregnancy outcomes at a lower cost in a large cohort of pregnant women: the St. Carlos Gestational Diabetes Study. Diabetes care. 2014;37(9):2442-50.

43. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PM, Damm P, et al. The diagnosis of gestational diabetes mellitus: new paradigms or status quo? The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2012;25(12):2564-9.

44. Blumer I, Hadar E, Hadden DR, Jovanovic L, Mestman JH, Murad MH, et al. Diabetes and pregnancy: an endocrine society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2013;98(11):4227-49.

45. Kaaja R, Laivuori H, Laakso M, Tikkanen MJ, Ylikorkala O. Evidence of a state of increased insulin resistance in preeclampsia. Metabolism: clinical and experimental. 1999;48(7):892-6.

46. Sacks DA, Hadden DR, Maresh M, Deerochanawong C, Dyer AR, Metzger BE, et al. Frequency of gestational diabetes mellitus at collaborating centers based on IADPSG consensus panel-recommended criteria: The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. Diabetes care. 2012;35(3):526-8.

47. Balaji V, Madhuri BS, Ashalatha S, Sheela S, Suresh S, Seshiah V. A1C in gestational diabetes mellitus in Asian Indian women. Diabetes care. 2007;30(7):1865-7.

48. Zhu WW, Yang HX, Wei YM, Yan J, Wang ZL, Li XL, et al. Evaluation of the value of fasting plasma glucose in the first prenatal visit to diagnose gestational diabetes mellitus in china. Diabetes care. 2013;36(3):586-90.

49. Zhang F, Dong L, Zhang CP, Li B, Wen J, Gao W, et al. Increasing prevalence of gestational diabetes mellitus in Chinese women from 1999 to 2008. Diabetic medicine: a journal of the British Diabetic Association. 2011;28(6):652-7.

50. Kaaja RJ, Greer IA. Manifestations of chronic disease during pregnancy. Jama. 2005;294(21):2751-7.

51. Getahun D, Nath C, Ananth CV, Chavez MR, Smulian JC. Gestational diabetes in the United States: temporal trends 1989 through 2004. American journal of obstetrics and gynecology. 2008;198(5): 525.e1-5.

52. Lawrence JM, Contreras R, Chen W, Sacks DA. Trends in the prevalence of preexisting diabetes and gestational diabetes mellitus among a racially/ethnically diverse population of pregnant women, 1999-2005. Diabetes care. 2008;31(5):899-904.

53. McCance DR. Diabetes in pregnancy. Best Practice & Research Clinical Obstetrics & Gynaecology. 2015;29(5):685-99.

54. Harris MI. Gestational diabetes may represent discovery of preexisting glucose intolerance. Diabetes care. 1988;11(5):402-11.

55. Kjos SL, Buchanan TA, Greenspoon JS, Montoro M, Bernstein GS, Mestman JH. Gestational diabetes mellitus: the prevalence of glucose intolerance and diabetes mellitus in the first two months postpartum. American journal of obstetrics and gynecology. 1990;163(1 Pt 1):93-8.

56. Gaudier FL, Hauth JC, Poist M, Corbett D, Cliver SP. Recurrence of gestational diabetes mellitus. Obstetrics and gynecology. 1992;80(5):755-8.

57. Moses RG. The recurrence rate of gestational diabetes in subsequent pregnancies. Diabetes care. 1996;19(12):1348-50.

58. Getahun D, Fassett MJ, Jacobsen SJ. Gestational diabetes: risk of recurrence in subsequent pregnancies. American journal of obstetrics and gynecology. 2010;203(5): 467.e1-6.

59. Shane B. Folate and vitamin B12 metabolism: overview and interaction with riboflavin, vitamin B6, and polymorphisms. Food and nutrition bulletin. 2008;29(2 Suppl): S5-16; discussion S7-9.

60. Martens JH, Barg H, Warren MJ, Jahn D. Microbial production of vitamin B12. Applied microbiology and biotechnology. 2002;58(3):275-85.

61. Glade MJ. Workshop on Folate, B12, and Choline. Sponsored by the Panel on Folate and other B vitamins of the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine, Washington, D.C., March 3-4, 1997. Nutrition (Burbank, Los Angeles County, Calif). 1999;15(1):92-6.

62. Fedosov SN, Fedosova NU, Berglund L, Moestrup SK, Nexo E, Petersen TE. Composite organization of the cobalamin binding and cubilin recognition sites of intrinsic factor. Biochemistry. 2005;44(9):3604-14.

63. Brada N, Gordon MM, Wen J, Alpers DH. Transfer of cobalamin from intrinsic factor to transcobalamin II. The Journal of nutritional biochemistry. 2001;12(4):200-6.

64. Shane B. Folate and vitamin B12 metabolism: overview and interaction with riboflavin, vitamin B6, and polymorphisms. Food and nutrition bulletin. 2008;29(2 Suppl): S5-16; discussion S7-9.

65. Herrmann W, Obeid R, Schorr H, Geisel J. The usefulness of holotranscobalamin in predicting vitamin B12 status in different clinical settings. Current drug metabolism. 2005;6(1):47-53.

66. Rush EC, Katre P, Yajnik CS. Vitamin B12: one carbon metabolism, fetal growth and programming for chronic disease. Eur J Clin Nutr. 2014;68(1):2-7.

67. Hunt A, Harrington D, Robinson S. Vitamin B12 deficiency. BMJ (Clinical research ed). 2014;349: g5226.

68. Mursleen MT, Riaz S. Implication of homocysteine in diabetes and impact of folate and vitamin B12 in diabetic population. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2017;11: S141-S6.

69. Kibirige D, Mwebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? Journal of diabetes and metabolic disorders. 2013;12(1):17.

70. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R. The prevalence of vitamin B (12) deficiency in patients with type 2 diabetes: a cross-sectional study. Journal of the American Board of Family Medicine: JABFM. 2009;22(5):528-34.

71. Liu Q, Li S, Quan H, Li J. Vitamin B12 status in metformin treated patients: systematic review. PloS one. 2014;9(6): e100379-e.

72. Nervo M, Lubini A, Raimundo FV, Faulhaber GA, Leite C, Fischer LM, et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. Revista da Associacao Medica Brasileira (1992). 2011;57(1):46-9.

73. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. Age and ageing. 2006;35(2):200-1.

74. Bell DS. Metformin-induced vitamin B12 deficiency presenting as a peripheral neuropathy. Southern medical journal. 2010;103(3):265-7.

75. Kumthekar AA, Gidwani HV, Kumthekar AB. Metformin associated B12 deficiency. The Journal of the Association of Physicians of India. 2012; 60:58-60.

76. Sukumar N, Venkataraman H, Wilson S, Goljan I, Selvamoni S, Patel V, et al. Vitamin B12 Status among Pregnant Women in the UK and Its Association with Obesity and Gestational Diabetes. Nutrients. 2016;8(12):768.

77. Krishnaveni GV, Hill JC, Veena SR, Bhat DS, Wills AK, Karat CL, et al. Low plasma vitamin B12 in pregnancy is associated with gestational 'diabesity' and later diabetes. Diabetologia. 2009;52(11):2350-8.

78. Tarim E, Bagis T, Kilicdag E, Erkanli S, Aslan E, Sezgin N, et al. Elevated plasma homocysteine levels in gestational diabetes mellitus. Acta obstetricia et gynecologica Scandinavica. 2004;83(6):543-7.

79. Guven MA, Kilinc M, Batukan C, Ekerbicer HC, Aksu T. Elevated second trimester serum homocysteine levels in women with gestational diabetes mellitus. Archives of gynecology and obstetrics. 2006;274(6):333-7.

80. Seghieri G, Breschi MC, Anichini R, De Bellis A, Alviggi L, Maida I, et al. Serum homocysteine levels are increased in women with gestational diabetes mellitus. Metabolism: clinical and experimental. 2003;52(6):720-3.

81. Idzior-Walus B, Cyganek K, Sztefko K, Seghieri G, Breschi MC, Walus-Miarka M, et al. Total plasma homocysteine correlates in women with gestational diabetes. Archives of gynecology and obstetrics. 2008;278(4):309-13.

82. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28(7):412-9.

83. Gestational diabetes mellitus. Diabetes care. 2004;27 Suppl 1: S88-90.

84. Langer O, Yogev Y, Most O, Xenakis EM. Gestational diabetes: the consequences of not treating. American journal of obstetrics and gynecology. 2005;192(4):989-97.

85. Tarim E, Yigit F, Kilicdag E, Bagis T, Demircan S, Simsek E, et al. Early onset of subclinical atherosclerosis in women with gestational diabetes mellitus. Ultrasound in obstetrics & gynecology: the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 2006;27(2):177-82.

86. Eades CE, Cameron DM, Evans JMM. Prevalence of gestational diabetes mellitus in Europe: A meta-analysis. Diabetes research and clinical practice. 2017; 129:173-81.

87. Finer S, Saravanan P, Hitman G, Yajnik C. The role of the one-carbon cycle in the developmental origins of Type 2 diabetes and obesity. Diabetic medicine: a journal of the British Diabetic Association. 2014;31(3):263-72.

88. Adaikalakoteswari A, Jayashri R, Sukumar N, Venkataraman H, Pradeepa R, Gokulakrishnan K, et al. Vitamin B12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes. Cardiovascular diabetology. 2014; 13:129.

89. Meigs JB, Jacques PF, Selhub J, Singer DE, Nathan DM, Rifai N, et al. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham offspring study. Diabetes care. 2001;24(8):1403-10.

90. Kothekar MA. Homocysteine in cardiovascular disease: a culprit or an innocent bystander? Indian journal of medical sciences. 2007;61(6):361-71.

91. Taneja S, Bhandari N, Strand TA, Sommerfelt H, Refsum H, Ueland PM, et al. Cobalamin and folate status in infants and young children in a low-to-middle income community in India. The American journal of clinical nutrition. 2007;86(5):1302-9.

92. Walker MC, Smith GN, Perkins SL, Keely EJ, Garner PR. Changes in homocysteine levels during normal pregnancy. American journal of obstetrics and gynecology. 1999;180(3 Pt 1):660-4.
93. de la Calle M, Usandizaga R, Sancha M, Magdaleno F, Herranz A, Cabrillo E. Homocysteine, folic acid and B-group vitamins in obstetrics and gynaecology. European journal of obstetrics, gynecology, and reproductive biology. 2003;107(2):125-34.
94. Reaven GM. Role of insulin resistance in human disease (syndrome X): an expanded definition. Annual review of medicine. 1993; 44:121-31.
95. Paolisso G, Gambardella A, Tagliamonte MR, Saccomanno F, Salvatore T, Gualdiero P, et al. Does free fatty acid infusion impair insulin action also through an increase in oxidative stress? The Journal of clinical endocrinology and metabolism. 1996;81(12):4244-8.
96. Yajnik CS, Deshpande SS, Lubree HG, Naik SS, Bhat DS, Uradey BS, et al. Vitamin B12 deficiency and hyperhomocysteinemia in rural and urban Indians. The Journal of the Association of Physicians of India. 2006; 54:775-82.
97. Selhub J, Morris MS, Jacques PF. In vitamin B12 deficiency, higher serum folate is associated with increased total homocysteine and methylmalonic acid concentrations. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(50):19995-20000.
98. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, et al. Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. Diabetologia. 2008;51(1):29-38.
99. Knight BA, Shields BM, Brook A, Hill A, Bhat DS, Hattersley AT, et al. Lower Circulating B12 Is Associated with Higher Obesity and Insulin Resistance during Pregnancy in a Non-Diabetic White British Population. PloS one. 2015;10(8): e0135268.
100. United States Department of Agriculture. USDA Table of Nutrient Retention Factors, Release 6. Available online: https://www.ars.usda.gov/ARSUserFiles/80400525/Data/retn/retn06.pdf (accessed on 5 November 2016).
101. Reinstatler L, Qi YP, Williamson RS, Garn JV, Oakley GP, Jr. Association of biochemical B (1)(2) deficiency with metformin therapy and vitamin B (1)(2) supplements: The National Health and Nutrition Examination Survey, 1999-2006. Diabetes care. 2012;35(2):327-33.
102. Chambers JC, Ueland PM, Obeid OA, Wrigley J, Refsum H, Kooner JS. Improved vascular endothelial function after oral B vitamins: An effect mediated through reduced concentrations of free plasma homocysteine. Circulation. 2000;102(20):2479-83.
103. Siddiqua TJ, Ahmad SM, Ahsan KB, Rashid M, Roy A, Rahman SM, et al. Vitamin B12 supplementation during pregnancy and postpartum improves B12 status of both mothers and infants but

vaccine response in mothers only: a randomized clinical trial in Bangladesh. European journal of nutrition. 2016;55(1):281-93.

104. Duggan C, Srinivasan K, Thomas T, Samuel T, Rajendran R, Muthayya S, et al. Vitamin B-12 supplementation during pregnancy and early lactation increases maternal, breast milk, and infant measures of vitamin B-12 status. The Journal of nutrition. 2014;144(5):758-64.

105. Chandyo RK, Ulak M, Kvestad I, Shrestha M, Ranjitkar S, Basnet S, et al. The effects of vitamin B12 supplementation in pregnancy and postpartum on growth and neurodevelopment in early childhood: Study Protocol for a Randomized Placebo Controlled Trial. 2017;7(8): e016434.

106. Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E. Homocysteine-lowering therapy and stroke risk, severity, and disability: additional findings from the HOPE 2 trial. Stroke. 2009;40(4):1365-72.

107. WHO Guidelines Approved by the Guidelines Review Committee. WHO Recommendations on Antenatal Care for a Positive Pregnancy Experience. Geneva: World Health Organization Copyright (c) World Health Organization 2016.; 2016.

SUPPLEMENTARY MATERIAL:

Supplementary table 1: Exclusion/Inclusion criteria

Exclusion criteria:

a. Not relevant with the subject
b. Not extractable data
c. Pregnant women treated already with drugs for GDM
d. Non-English language
e. Conducted in animals or in vitro

Inclusion criteria:

a. Population of the study includes pregnant women
b. Data on vitamin B12 concentration for GDM and non-GDM women
c. Outcome: GDM

Supplementary table 2. Full-text articles excluded from data synthesis.

Study	**Year**	**Reasons for exclusion**	**Number of studies**
Adaikalakoteswari A. et al.	2017	Study in vitro	2
Sinclair KD et al.	2007		
Knight BA et al.	2015	No data on women with GDM	1
Paez Pumar JI.	1959	Non-English language	1

Supplementary table 3. Study quality as assessed by the Newcastle-Ottawa scale.

Id	First author, Year	Selection	Comparability	Exposure / Outcome	Overall quality
1.	Sukumar, 2016	4	2	2	Good
2.	Krishnaveni, 2009	4	2	2	Good
3.	Tarim, 2004	4	2	2	Good
4.	Guven , 2006	4	2	2	Good
5.	Seghieri, 2003	4	2	2	Good
6.	Idzior-Walus, 2008	4	2	2	Good

According to the Newcastle-Ottawa scale, a study can be awarded a maximum of four stars for the selection category, a maximum of two stars for the comparability category and a maximum of three stars for the outcome/exposure category.